JCB Journal of Cell Biology

VIEWPOINT

# Lysosomal acidification through membrane contacts with mitochondria

Zhiqi Tian[1], Yujie Sun[1], and Jiajie Diao[2]

**Recent work from two independent studies has uncovered mitochondria as a previously unrecognized proton source driving lysosomal acidification. In this Viewpoint, we summarize these findings, examine the associated proteins and functional mechanisms, and discuss their relevance to disease.**

## Introduction

Lysosomal acidification is essential for the activity of lysosomal hydrolases, which underpins the organelle's digestive function (Mindell, 2012). The lysosomal lumen is normally maintained at a pH of ∼4.5–5.0, an acidic environment that is required for the proper folding and catalytic activity of more than 60 hydrolytic enzymes, including proteases, lipases, and glycosidases (Mindell, 2012). Disruption of this acidic environment impairs substrate degradation and autophagic flux, and has been linked to numerous pathological conditions, including neurodegenerative diseases and lysosomal storage disorders.

In the classical model of lysosomal acidification, the lysosomal V-ATPase pumps protons from the cytosol into the lysosomal lumen against a steep concentration gradient, using energy derived from ATP hydrolysis (Mindell, 2012). This multi-subunit proton pump is thought to be the primary, and in many models the sole, mechanism responsible for establishing and maintaining the pH gradient across the lysosomal membrane. Therefore, lysosomes would be acidified evenly across entire cells, since V-ATPase activity and cytosolic proton availability would be expected to be relatively uniform throughout the cytoplasm.

However, this hypothesis cannot fully account for the heterogeneity of lysosomal pH observed across the cytosol in live cells, where individual lysosomes within the same cell can display markedly different luminal pH values depending on their subcellular localization and functional state (Johnson et al., 2016). Such spatial heterogeneity is difficult to reconcile with a model in which a single, ubiquitously distributed pump is the sole determinant of acidification. Moreover, the cytosol itself is a poorly buffered, near-neutral compartment (pH ∼7.2), and the limited availability of free protons in the cytosol would be insufficient to continuously sustain the pumping activity required for lysosomal acidification over time, particularly given the high turnover and dynamic fusion–fission behavior of the lysosomal compartment (Liu et al., 2026). Therefore, there must be another major proton source, beyond cytosolic free protons alone, that contributes to and sustains lysosomal acidification.

### Mitochondria acidify lysosomes

Super-resolution imaging in live cells showed that mitochondria can directly interact with lysosomes besides degradative interactions (Chen et al., 2018; Leisten et al., 2023; Wong et al., 2018). Most recently, two independent studies revealed that mitochondria are the major proton donor for lysosomal acidification through these direct contacts between mitochondria and lysosomes (Fig. 1 A) (Liu et al., 2026; Tian et al., 2026), challenging the traditional view that acidification relies only on free cytosolic protons. The first study identified mitochondria as a proton source for lysosomal acidification at mitochondria–lysosome contact (MLC) sites in human cell lines (Tian et al., 2026). Using two newly designed molecular probes—AN-PZ, which reports lysosomal content digestion via viscosity-sensitive fluorescence, and PP-1, which reports mitochondrial membrane polarity as a proxy for proton flux—the authors show that lysosomes in contact with mitochondria exhibit enhanced content degradation and increased acidification compared with lysosomes without such contacts or those contacting the endoplasmic reticulum. Time-lapse super-resolution imaging captured real-time proton transfer from mitochondria to lysosomes during MLC formation, and this transfer was blocked by electron transport chain inhibitors (antimycin A, rotenone) and a V-ATPase inhibitor (bafilomycin A1), implicating mitochondrial proton pumping and lysosomal V-ATPase in the mechanism. Using an optogenetic system to artificially induce MLCs with blue light, the researchers demonstrated that forcing mitochondria and lysosomes into proximity increases lysosomal acidity and enhances content digestion, including under disease-mimicking conditions induced by U18666A, an intracellular cholesterol transport inhibitor resulting in cholesterol accumulation. Overall, the study reveals a previously unknown physiological role for MLCs as sites of interorganelle proton exchange that supports lysosomal digestive function, with

[1]Department of Chemistry, University of Cincinnati, Cincinnati, OH, USA; [2]Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH, USA.

Correspondence to Jiajie Diao: jiajie.diao@uc.edu.

© 2026 Tian et al. This article is distributed under the terms as described at https://rupress.org/pages/terms102024/.



Check for updates

https://doi.org/10.1083/jcb.202606052



Downloaded from rupress.org/jcb/article-pdf/225/12/e202606052/2043253/jcb_202606052.pdf by guest on 05 November 2026

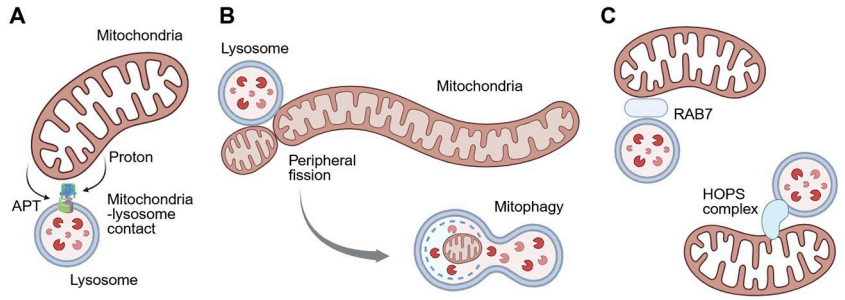



Figure 1. **Mitochondria-Lysosome contact sites. (A)** Through MLCs, mitochondria provide ATP to drive the movement of V-ATPase that pumps mitochondrial protons for lysosomal acidification. **(B)** MLCs can promote peripheral mitochondrial fission, facilitating subsequent mitophagy and thereby contributing to mitochondrial quality control. **(C)** Both RAB7 and the HOPS complex have been proposed to contribute to the formation of MLCs. HOPS, homotypic fusion and protein sorting.

implications for diseases involving impaired lysosomal acidification.

The second study identified that mitochondria supply protons to lysosomes/vacuoles through direct membrane contact sites (Liu et al., 2026). Using yeast and human cell models, the authors show that mitochondria–vacuole (Mito-Vac) contacts and MLCs—mediated by proteins like Vam6, Ypt7, and RAB7a—enable proton transfer from the mitochondrial electron transport chain to acidify these organelles, an effect confirmed through genetic manipulation, molecular simulations, and an engineered synthetic linker that enhances contact and acidification. During yeast replicative aging, these contacts decline in mother cells (driving vacuole deacidification) but are restored in daughter cells via a Bni1/actin cable-dependent and Mob2-Vam6 mechanism, enabling rejuvenation; artificially maintaining Mito-Vac contacts extended yeast replicative lifespan by ~20%. In human cellular senescence, reduced MLCs similarly cause lysosomal dysfunction but restoring these contacts with an engineered linker rescues autophagic flux and substantially suppresses the senescence-associated secretory phenotype, including inflammatory cytokines. Overall, the study establishes a conserved mitochondria-to-lysosome/vacuole proton-transfer axis as a key regulator of organelle acidification, aging, and senescence, suggesting that restoring organelle contacts could be a therapeutic strategy for age-related lysosomal dysfunction.

## Self-limiting proton transfer

One intriguing aspect of mitochondria-induced lysosomal acidification is that it appears to be inherently self-limiting. As electrons pass along the electron transport chain, the energy released is used to pump protons from the mitochondrial matrix, across the inner membrane, into the intermembrane space (IMS) (Bonora et al., 2012). This proton gradient across the inner membrane is essential for ATP production via oxidative phosphorylation (Bonora et al., 2012), whereas ATP, in turn, is required to power the V-ATPase proton pumps that acidify lysosomes (Fig. 1 A) (Mindell, 2012). This creates an interdependence between the two processes, since mitochondrial protons directly transferred to lysosomes are protons that are simultaneously unavailable to drive ATP synthase. Therefore, once proton transfer from mitochondria to lysosomes becomes sufficient to compromise mitochondrial ATP production, lysosomal proton pumping—and consequently further acidification—is expected to slow. In this way, the system behaves as a negative feedback loop, in which the very process of lysosomal acidification generates a constraint that limits its own continuation once mitochondrial energy output begins to falter. This self-limiting mechanism would prevent excessive depletion of mitochondrial protons, thereby protecting mitochondria from potentially catastrophic dysfunction. Moreover, because MLCs are important for lysosomal acidification, the heterogeneity of lysosomal pH may arise from their uneven distribution, with more acidic lysosomes located closer to the nucleus.

## Involvement in mitochondrial quality control

Another intriguing aspect of MLCs is that lysosomes can induce mitochondrial fission at these contact sites, predominantly in peripheral regions of mitochondria, a spatial pattern that distinguishes this fission from the midzone divisions typically associated with mitochondrial biogenesis (Wong et al., 2018). A separate study showed that peripheral mitochondrial fission primarily serves a quality-control function by segregating damaged mitochondrial components for repair or removal, including through mitophagy (Kleele et al., 2021). Although many lysosome-induced fission events are not involved in mitophagy and another study suggests that endolysosomes enable local translation of mitochondrial fission factor to support peripheral fission (Fenton et al., 2024), this positional specificity suggests that MLC-induced fission is not a stochastic byproduct of organelle contact but rather a spatially targeted mechanism for isolating dysfunctional mitochondrial fragments. Because lysosomal acidification is essential for efficient mitophagic degradation, proton transfer from mitochondria to lysosomes at MLCs may coordinate these two processes: facilitating the excision of damaged mitochondrial segments while simultaneously preparing lysosomes for their subsequent digestion (Fig. 1 B). Viewed this way, the same contact site that triggers fission could also supply the acidifying protons needed downstream, linking the physical severing of damaged material to the biochemical readiness of the lysosome that will consume it. Such coupling would offer an elegant efficiency: rather than fission and lysosomal acidification occurring as independently regulated events, they would be mechanistically synchronized at the point of contact. This raises the possibility that MLCs function as integrated signaling hubs that couple mitochondrial quality control directly to lysosomal digestive capacity, rather than merely serving as passive sites of organelle apposition.

## Proteins involved in MLC formation

In the original study that identified MLCs, activation of lysosomal GTP-bound RAB7 was shown to promote MLC formation, whereas recruitment of the RAB7 GTPase-activating protein TBC1D15 to mitochondria by FIS1 was proposed to trigger RAB7 GTP hydrolysis and thereby promote contact release (Wong et al., 2018) (Fig. 1 C). Meanwhile, other studies have shown that the homotypic fusion and protein sorting

Downloaded from rupress.org/jcb/article-pdf/225/12/e202606052/2043253/jcb_202606052.pdf by guest on 05 November 2026

complex also contributes to bridging Mito-Vac contacts in yeast and MLCs in mammalian cells (González Montoro et al., 2018; Tian et al., 2026) (Fig. 1 C). However, unlike a recent study that identified a protein channel for material exchange between mitochondria and the nucleus, involving an interaction between the mitochondrial outer membrane protein voltage-dependent anion channel 1 (VDAC1) and the nuclear pore protein Ran-binding protein 2 (Menendez-Montes et al., 2026), none of the proteins described above is known to form a channel that could directly mediate proton transfer. Therefore, VDAC1 might also serve as a channel through which IMS protons exit the mitochondria and move toward lysosomes. Moreover, it is possible that mitochondrial VDAC1 interacts with a channel or proton-transporting protein on the lysosomal membrane, such as V-ATPase.

### Therapeutics

Given the close association between impaired lysosomal acidification and numerous diseases, these two studies open new avenues for therapeutic intervention by modulating membrane contact sites (Qiu et al., 2022). Rather than targeting lysosomal proton pumps or hydrolases directly, these approaches instead aim to restore acidification indirectly by manipulating the physical and functional coupling between mitochondria and lysosomes. Tian et al. demonstrated that optogenetic enhancement of MLCs increases lysosomal acidification and alleviates disease phenotypes resembling those of lysosomal storage disorders (Tian et al., 2026). This finding provides proof-of-concept evidence that artificially promoting the MLC is sufficient to restore lysosomal function even in the context of underlying disease-associated defects. Liu et al. further showed that antibody-mediated enhancement of MLCs counteracts their age-related decline and partially restores aging-associated phenotypes (Liu et al., 2026). Together, these studies suggest that MLCs are not merely a downstream consequence of cellular aging or disease but represent a tractable node whose targeted manipulation could yield broadly applicable therapeutic strategies. Finally, it is important to note that MLCs are highly dynamic processes that require precise on/off control. Constitutive tethering, as seen in Charcot–Marie–Tooth type 2B–associated RAB7 mutations, may therefore damage cells by causing excessive fission or lysosomal overacidification.

## Conclusion

The study of functional membrane contacts between different organelles is gaining increasing attention, and many new discoveries are expected to emerge in this rapidly developing field. Meanwhile, in the crowded environments inside cells, organelles constantly come into contact with one another as they move and jostle within the limited cytoplasmic space. Some of these encounters are purely incidental, arising simply from the high density of organelles and the random nature of intracellular motion. Others, however, reflect genuine physiological interactions, mediated by specific tethering proteins and signaling molecules that coordinate organelle function. Distinguishing between these two categories is inherently challenging, since both types of contact can appear morphologically similar under current imaging techniques. Therefore, developing new biosensors with functional readouts, such as changes in membrane viscosity at contact sites (Chen et al., 2020), will be essential for reliably distinguishing stochastic organelle collisions from functionally significant, protein-mediated interactions (Filadi et al., 2026).

### Acknowledgments

J. Diao is supported by the National Institutes of Health grant R35GM128837.

Author contributions: Zhiqi Tian: writing—original draft, review, and editing. Yujie Sun: funding acquisition and writing—review and editing. Jiajie Diao: conceptualization, funding acquisition, and writing—original draft.

Disclosures: The authors declare no competing interests exist.

## References

Bonora, M., et al. 2012. *Purinergic Signal.* https://doi.org/10.1007/s11302-012-9305-8
Chen, Q., et al. 2020. *Nat. Commun.* https://doi.org/10.1038/s41467-020-20067-6
Chen, Q., et al. 2018. *Small.* https://doi.org/10.1002/smll.201802166
Fenton, A.R., et al. 2024. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-024-01544-2
Filadi, R., et al. 2026. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-026-02003-w
González Montoro, A., et al. 2018. *Dev. Cell.* https://doi.org/10.1016/j.devcel.2018.05.011
Johnson, D.E., et al. 2016. *J. Cell Biol.* https://doi.org/10.1083/jcb.201507112
Kleele, T., et al. 2021. *Nature.* https://doi.org/10.1038/s41586-021-03510-6
Leisten, E.D., et al. 2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202305032
Liu, Q., et al. 2026. *Mol. Cell.* https://doi.org/10.1016/j.molcel.2026.05.004
Menendez-Montes, I., et al. 2026. *Nature.* https://doi.org/10.1038/s41586-026-10588-3
Mindell, J.A. 2012. *Annu. Rev. Physiol.* https://doi.org/10.1146/annurev-physiol-012110-142317
Qiu, K., et al. 2022. *Nat. Commun.* https://doi.org/10.1038/s41467-022-31970-5
Tian, Z., et al. 2026. *Cell Rep.* https://doi.org/10.1016/j.celrep.2026.117112
Wong, Y.C., et al. 2018. *Nature.* https://doi.org/10.1038/nature25486

Downloaded from rupress.org/jcb/article-pdf/225/12/e202606052/2043253/jcb_202606052.pdf by guest on 05 November 2026